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Original Article

A C-terminal truncated mutation of *spr-3* gene extends lifespan in *Caenorhabditis elegans*

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The lifespan of *Caenorhabditis elegans* is determined by various genetic and environmental factors. In this paper, *spr-3*, a *C. elegans* homologous gene of the mammalian neural restrictive silencing factor (*NRSF/REST*), is reported to be an important gene regulating lifespan of *C. elegans*. A deletion mutation of *spr-3*, *spr-3(ok2525)*, or RNAi inhibition of *spr-3* expression led to the short lifespan phenotype in *C. elegans*. However, a nonsense mutation of *spr-3*, *spr-3(by108)*, increased the lifespan by 26% when compared with that of wild-type nematode. The *spr-3(by108)* also showed increased resistance to environmental stress. The *spr-3(by108)* mutated gene encodes a C-terminal truncated protein with a structure comparable with the REST4, a splice variant of the NRSF/REST in mammalian. The long lifespan phenotype of *spr-3(by108)* mutant is confirmed as a gain of function and dependent on normal functions of *daf-16* and *glp-1*. The lifespan of the *spr-3(by108)* can be synergistically enhanced by inducing a mutation in *daf-2*. Quantitative polymerase chain reaction results showed that the expression of *daf-16* as well as its target gene *sod-3*, *mtl-1*, and *sip-1* was up-regulated in the *spr-3(by108)* mutant. These results would be helpful to further understand the complex function of *NRSF/REST* gene in mammalian, especially in the aging process and longevity determination.

Keywords NRSF/REST; *spr-3*; *daf-2*; *daf-16*; lifespan

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Introduction

Neuron-restrictive silencer factor (NRSF), also known as repressor element-1 (RE-1) silencing transcription factor (REST), was initially identified as a key transcription repressor during the process of mouse embryo development and neurogenesis. NRSF/REST belongs to the family of Kruppel-type zinc-finger transcription factor that is conserved in mouse and human. It recognizes a 21-nt consensus DNA sequence called neuronal restrictive silencing element (NRSE) or RE-1 and works as a hub for interacting with the co-repressors CoREST or/and mSin3A [1,2], which in turn recruits histone modifiers such as HDAC1/2 to modify epigenetic status of chromatin structure and in most case to down-regulate the local gene expression [3]. Thousands of genes have been verified as NRSF/REST target genes in human and mouse genome by either bioinformatics prediction or by chip-sequencing analysis. Many of them are found not to be as just neuronal-specific genes, suggesting that they may function in other biological processes. There are various alternative splicing isoforms in *NRSF/REST* gene transcripts, which increase the complexity of the gene function. For example, REST4, a C-terminal truncated isoform of NRSF/REST transcript, was reported to act as an antagonist against the function of full-length NRSF/REST protein in mouse [4]. More and more studies indicated that NRSF/REST plays a role in the physiological and pathological processes *in vivo*, in addition to its functions in early embryonic development and neurogenesis, such as in cardiovascular development and carcinogenesis [5]. However, as a pivotal epigenetic regulator, the function of NRSF/REST in aging and longevity has not been well evaluated.

The relationship between epigenetics and aging was proposed in 1967 [6]. Since then, epigenetic status, especially DNA methylation and chromatin structure modifications in the aging process has been widely studied. It is now well accepted that the epigenetic modification plays an important role in regulation of the longevity and aging in many species [7–9]. A recent study showed that the phenotype of longevity in *Caenorhabditis elegans* caused by histone H3 lysine 4 trimethylation (H3K4me3) complex deficiency can be transgenerationally inherited to the wild-type descendants [10]. Thus, studies of the genetic requirements for epigenetic regulation of lifespan are an important approach to understand the molecular mechanisms of aging process and longevity determination.

C. elegans is widely used as a model organism in the field of aging research owing to its relative short lifespan, conserved signal transduction pathways, and abundant mutant resources. *spr-3* and *spr-4* were reported as *NRSF/REST* homology counterparts in *C. elegans* genome. Mutation of *spr-3* or *spr-4* was found to rescue deficiency phenotype of egg laying caused by *sel-12* gene mutagenesis, the homolog gene of the mammalian presenilin in *C. elegans* [11].

In this study, we investigated the effect of *spr-3* mutation on the lifespan of *C. elegans*. There are two *spr-3* gene mutants available at Caenorhabditis Genetics Center (CGC; Minneapolis, USA). We found that deficiency of *spr-3* function affects the lifespan of *C. elegans* in a different way dependent on the type of mutation. The *spr-3(ok2525)*, a deletion mutation in 5′-terminal region of *spr-3* gene including the translation initiation site, exhibits a shorter lifespan than that of wild type. This phenotype was confirmed by RNAi inhibition of *spr-3* in *C. elegans*. Surprisingly, another *C. elegans* strain *spr-3(by108)* harboring a mutant *spr-3* gene that encoded a C-terminal truncated protein showed a longer lifespan than that of wild type. The lifespan extension of *spr-3(by108)* can be found in heterozygote and inhibited by the RNAi treatment, indicating a dominant effect of this truncated mutation product. Meanwhile, the phenotype of *spr-3(by108)* is abolished by the mutation of *daf-16* or *glp-1* but not *daf-2* deficiency. These results indicated that the role of *spr-3* in aging process is complex.

Materials and Methods

Strains

Nematode strains used in this study were either provided by the CGC, or derived from CGC-supplied strains in our laboratory. The wild-type strain used is N2. The mutant strains were *spr-3(by108)*, *spr-3(ok2525)*, *daf-2(e1370)*, *daf-16(mu86)*, *glp-1(e2141)*, and *rrf-3(pk1426)*. The following double-mutant strains, *daf-16(mu86)*;*spr-3(by108)*, *daf-2(e1370)*;*spr-3(by108)*, and *glp-1(e2141)*;*spr-3(by108)* were raised using standard genetic methods. All *spr-3* mutants used in this study were backcrossed with N2 for five generations to eliminate the genetic background difference before experiments.

Worm cultivation

Nematodes were cultivated at 21°C on nematode growth media (NGM) plates with *Escherichia coli* OP50. For culture of *daf-2(e1370)*, *daf-2(e1370)*;*spr-3(by108)*, and their wild-type control, nematodes were cultivated at 16°C for 3 days and then transferred to 21°C for the desired stage of development.

Lifespan assays

Adult hermaphrodites were transferred onto fresh NGM plates, allowed to lay eggs for 4 h and then removed. Age-synchronized eggs were collected and picked to fresh RNAi or OP50 plates and allowed to grow for 3 days until they became young adults. Lifespan measurement was carried out on RNAi plates or OP50 plates with 5-fluorodeoxyuridine (Sigma, St Louis, USA) to a final concentration of 20 μm. Lifespan assays were performed as previously described [12]. Living worms were scored every day or every other day, and those that failed to respond to a gentle prodding with a platinum wire were scored as dead. Animals that bagged, exploded, or crawled off the plate were considered as censored. We defined the date when we transferred the young adult worms as Day 0 of adult lifespan. All the lifespan experiments were repeated at least three independent times. The acceptance level for statistical significance was $P < 0.05$.

Stress assays

For the paraquat tolerance assay, synchronized Day 3 adult worms were collected and washed by M9 buffer for three times to remove the OP50 bacteria. Approximately 30 adults were suspended into each well of a 48-well culture plate containing 160 μl of 100 or 50 mM paraquat (Supelco, Bellefonte, USA) in M9 buffer [12]. The plates were incubated at 21°C for certain time. The survived nematodes were counted at different time points. The $K_2Cr_2O_7$ (Sangon Biotech, Shanghai, China) and $CdCl_2$ (Sangon Biotech) tolerance assays were carried out following the same way as with paraquat except that 10 mM $K_2Cr_2O_7$ or 20 mM $CdCl_2$ was used instead of paraquat.

For the heat-shock assay, synchronized Day 3 adult worms were prepared as described above and then shifted to 33°C on OP50-NGM plates for certain time. The survived worms were counted [12].

Each experiment was repeated for at least three times.

Dauer assay

For dauer assay, the synchronized eggs were allowed to develop at 21 or 25°C for 120 h. The worms were treated with 1% sodium dodecyl sulfate (SDS; Sangon Biotech) for 15 min, and the survived larvae were considered as dauer nematodes [13].

RNA isolation and real-time polymerase chain reaction

Total RNA of each kind of nematode strain was isolated with Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol and the literature [13]. Then RNA was reversely transcribed to cDNA by using reverse transcriptase (MMLV; Promega, Madison, USA) according to the manufacturer's instructions. Quantitative real-time

Table 1 Primers used for quantitative PCR

Gene	Sequence (5′→3′)
act-4	GCCACCGCTGCCTCCTCATC (F)
	CCGGCAGACTCCATACCCAAGAAG (R)
daf-2	ACAGCCAAGATATTC CCAAGACGA (F)
	ACGGCCTCCAATTACACGAAGAT (R)
daf-16	GAAAGAGCTCGTGGTGGGTTATTA (F)
	TCCGCGGCGAGATTTTTC (R)
sod-3	GACATCACTATTGCGGTTCA (F)
	TCTGGGCGGACATTTTT (R)
mtl-1	TGTGAGGAGGCCAGTGAGA (F)
	TTAATGAGCCGCAGCAGTT (R)
sip-1	CGAGCACGGGTTCAGCAAGAG (F)
	CAGCGTGTCCAGCAGAAGTGTG (R)

F, forward primer; R, reverse primer.

polymerase chain reaction (qRT-PCR) was performed using the 7300 Real-Time PCR System with SYBR-Green I as fluorescent dye (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. The *act-4* was used as the internal control to normalize the expression level of target genes. Primers used for qRT-PCR experiments are shown in **Table 1**.

RNAi inhibition

The RNAi sequence of *spr-3* was amplified from *C. elegans* genomic DNA by PCR using ExTaq polymerase (TaKaRa, Dalian, China). The sequence of primers is as follows: forward primer, 5′-TGCTCTAGATCGAGCGAGAAGC AGATGTAT-3′, and reverse primer, 5′-GGGGTACCTCA CGGTGGAGACGGAACT-3′. The PCR product contained part of *spr-3* exon2, whole exon3, exon4 and part of exon5. The PCR product was digested with *Xba*I (NEB, Ipswich, USA) and *Kpn*I (NEB) that was designed in the sequence of primers and inserted into L4440 vector [14]. The recombined plasmid was transformed into the HT115(DE3) bacterial competent cells [15]. The transformed colonies were picked up and the recombinant plasmid was extracted and verified by sequencing.

RNAi plates were prepared with NGM with 50 μg/ml ampicillin and 1 mM isopropyl β-D-1-thiogalactopyranoside. After being poured, the plates were kept at room temperature for 3 days. RNAi plasmid transformed bacteria were grown overnight at 37°C in Luria–Bertani (LB) media with 50 μg/ml ampicillin and 12.5 μg/ml tetracycline. The overnight cultures were diluted (1 : 30) with LB containing 50 μg/ml ampicillin and grown at 37°C until OD600 = 1.0. About 100 μl of the bacterial suspension was seeded onto the RNAi plate. The seeded plates were kept at room temperature for 3 days before being used in the RNAi experiment.

Statistical analysis

Data are presented as the mean ± SEM. Groups were compared using one-way analysis of variance (ANOVA) followed by *t*-test. Differences among multiple means were assessed by one-way ANOVA followed by Bonferroni correction. Statistical software OriginPro 7.5 was used (http://www.originlab.com/).

Results

Mutation of *spr-3* affects lifespan in *C. elegans*

Two *C. elegans* strains with mutant alleles (*ok2525* and *by108*) of *spr-3* were verified by PCR and sequencing (**Fig. S1**). Mutant of *spr-3(ok2525)* has a 595-bp deletion containing the translation initiation site and downstream N-terminal 138 amino acids. The other mutant, *spr-3(by108)*, contains a nonsense mutation in which the 465th amino acid codon CAG was changed into a stop codon TAG leading to an early termination. The *spr-3(ok2525)* mutant exhibited a significant shorter lifespan than wild-type worms [**Fig. 1(A)** and **Table S1**], while mutant *spr-3(by108)* showed an increased lifespan by 26% compared with that of wild-type nematode [**Fig. 1(B)** and **Table S1**].

To further confirm the role of *spr-3* in longevity, *spr-3* was knockdown by RNAi in *C. elegans* with a background of *rrf-3* mutation *(pk1426)* [16], which is sensitive to RNAi treatment. Result from qRT-PCR showed that *spr-3* mRNA level in *spr-3* RNAi-treated worms was down-regulated by 69% compared with the control group. *spr-3* RNAi treatment led to a shorter lifespan in *rrf-3 (pk1426) C. elegans* suggesting that *spr-3(ok2525)* acted as a loss of function mutation [**Fig. 1(C)** and **Table S1**]. However, RNAi treatment also impaired the long life phenotype of the mutant *spr-3(by108);rrf-3(pk1426)* indicating a gain of function mutation in mutant *spr-3(by108)* [**Fig. 1(D)** and **Table S1**]. The dominant effect of *spr-3* C-terminal truncated mutation in *spr-3(by108)* was further confirmed by the result that heterozygous *spr-3(by108)* showed a longer lifespan but without a significant difference from that of homozygous [**Fig. 1(E)** and **Table S1**]. The homozygous *spr-3(by108)* without further lifespan extension may be explained by the ceiling effect of mutant gene product. Overall, our results demonstrated that the function of *spr-3* gene is involved in aging process and longevity determination in *C. elegans*.

The long lifespan phenotype of *spr-3(by108)* is dependent on *daf-16* function and normal germline signaling

The *daf-16/FOXO* is one of the most important and best characterized longevity determinants in *C. elegans* [17]. To investigate whether the long lifespan phenotype of the mutant *spr-3(by108)* is dependent on the function of *daf-16*,

we produced *daf-16(mu86);spr-3(by108)* double mutant. Our results showed that *daf-16(mu86);spr-3(by108)* has a lifespan indistinguishable from that of the *daf-16(mu86)* single mutant, which is significantly shorter than that of wild-type worms [**Fig. 2(A)** and **Table S2**], indicating that the long lifespan phenotype of *spr-3(by108)* requires the normal activity of *daf-16*.

It was reported that deficiency of germline signaling extends lifespan of *C. elegans* [18,19]. *glp-1(e2141)* is a germline-deficient mutant with an increasing lifespan [20]. To test whether the phenotype of *spr-3(by108)* is dependent on germline signaling, we produced *glp-1(e2141);spr-3 (by108)* double mutant. As shown in **Fig. 2(B)** and **Table S1**, both single mutants showed the phenotype of extended lifespan compared with wild type; however, there is no significant difference between the double-mutant and wild-type nematode. These results reveal a complex and interesting phenomenon, that is, the effect of *spr-3(by108)* mutation on the lifespan is dependent on the normal germline signaling and vice versa.

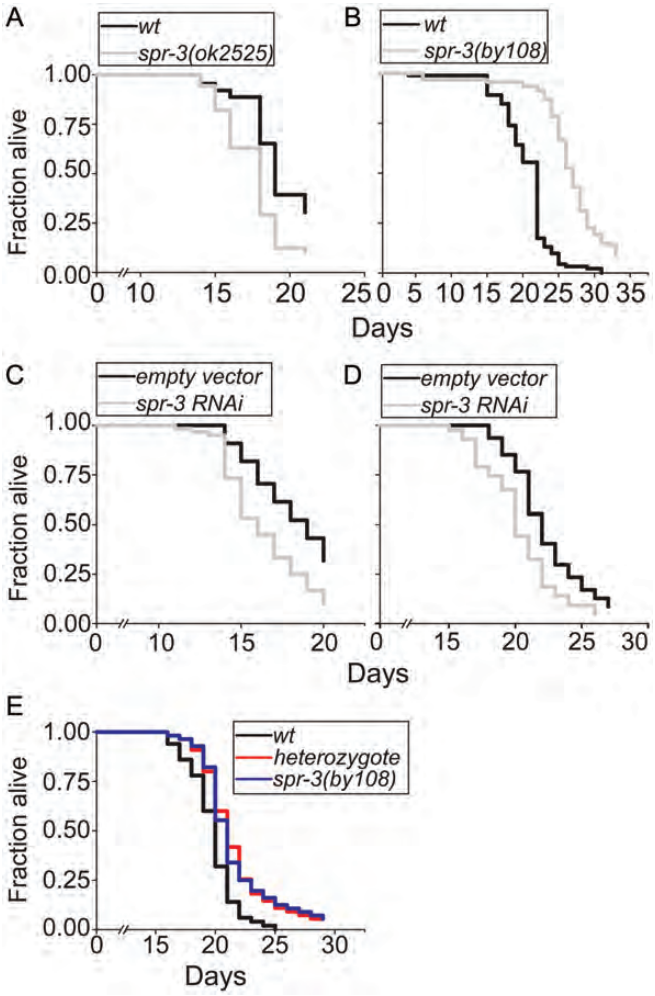

Figure 1 The effect of *spr-3* mutation on lifespan of *C. elegans* (A) The *spr-3(ok2525)* mutant showed a significant decreased lifespan than wild-type worms. Mean lifespan [days ± SEM (*n*)] of worms is as follows: wild type, 18.3 ± 0.23 ($n = 62$); *spr-3(ok2525)*, 17.2 ± 0.60 ($n = 80$). $P < 0.001$ (B) *spr-3(by108)* mutant showed significant increased lifespan. Mean lifespan for wild-type N2, 20.6 ± 0.4 ($n = 92$); for *spr-3(by108)*, 26.0 ± 0.5 *(n* = 85). $P < 0.00001$. (C) Inhibition of *spr-3* in *rrf-3(pk1426)* mutant by RNAi treatment decreased significantly its lifespan. Mean lifespan [days ± SEM (*n*)] of *rrf-3(pk1426)* is as follows: on vector RNAi, 20.3 ± 0.5 ($n = 38$); on spr-3 RNAi, 17.8 ± 0.5 ($n = 43$). $P < 0.01$. (D) Extended long lifespan phenotype could be eliminated by *spr-3* RNAi treatment in *rrf-3(pk1426);spr-3(by108)* double mutants. Mean lifespan [days ± SEM (*n*)] of *rrf-3(pk1426);spr-3(by108)*, is as follows: on vector RNAi, 21.9 ± 0.36 ($n = 43$); on spr-3 RNAi, 20.1 ± 0.40 ($n = 41$). $P <$ 0.01. (E) The heterozygous *spr-3 (by108)* showed a significant longer lifespan. Mean lifespan for wild-type N2 19.76 ± 0.27 ($n = 50$), for heterozygote *spr-3(by108)* was 22.19 ± 0.45 ($n = 52$), and for *spr-3(by108)* was 22.19 ± 0.43 ($n = 53$). $P < 0.01$. wt, wild type.

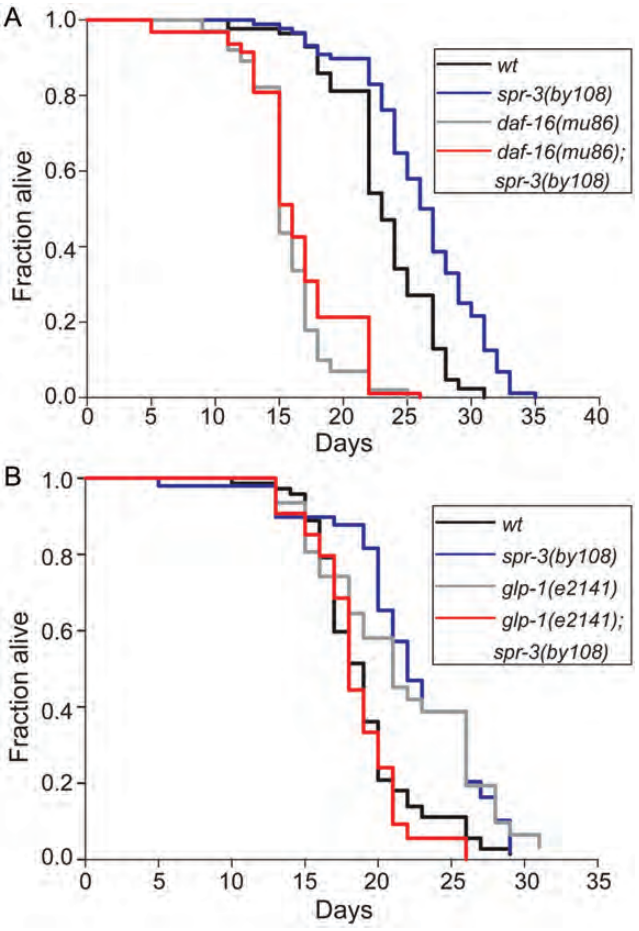

Figure 2 The long lifespan of *spr-3(by108)* by *daf-16* and germline signaling pathways The long lifespan phenotype of *spr-3(by108)* is dependent on the normal function of *daf-16* (A), and germline signaling in *C. elegan* (B). Mean life span [days ± SEM (*n*)] of worms is as follows: wild type, 23.1 ± 0.45 ($n = 85$); *spr-3(by108)*, 26.2 ± 0.49 ($n = 88$), $P < 0.0001$. *daf-16(mu86),* 15.7 ± 0.29 ($n = 101$), $P < 0.00001$; *daf-16(mu86);spr-3(by108)*, 16.4 ± 0.40 ($n = 94$), $P < 0.00001$; wild type, 18.9 ± 0.41 ($n = 71$); *spr-3(by108)*, 22.2 ± 0.72 ($n = 48$), $P <$ 0.00001; *glp-1(e2141)*, 21.3 ± 0.99 ($n = 30$) $P < 0.01$; and *glp-1(e2141);spr-3(by108)*, 18.48 ± 0.40 ($n = 54$), $P = 0.5$. wt, wild type.

The mutation of *spr-3(by108)* significantly extends the lifespan of *daf-2* mutant

To test whether the determination of *C. elegans* lifespan by mutation in *spr-3(by108)* is functionally dependent on the IIS pathway, a well-established upstream regulator of *daf-16* [21], we examined the genetic interaction between *spr-3(by108)* and the insulin receptor *daf-2*, the major component of the IIS pathway. The expression of *daf-2* is down-regulated in *spr-3(by108)* mutant indicating a possible mechanism of *spr-3(by108)* for lifespan regulation [**Fig. 3(A)**]. However, the average lifespan of the double-mutant *daf-2(e1370);spr-3(by108)* was 54 days that is much longer than that of each single mutant [**Fig. 3(B)** and **Table S3**]. This indicated that *spr-3(by108)* can synergistically interact with the deficient IIS pathway to increase the lifespan.

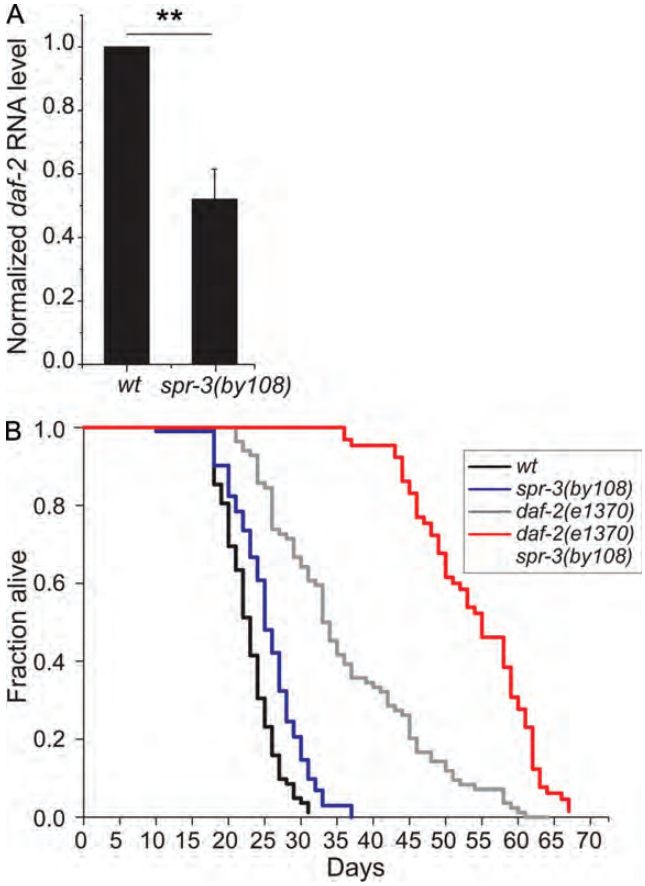

Figure 3 ***spr-3(by108)*** **can synergistically interact with the deficient IIS pathway to increase the lifespan** (A) The RNA levels of *daf-2* in wild-type and *spr-3(by108)* mutant were quantified using qRT-PCR. (B) The lifespan of *daf-2(e1370);spr-3(by108)* double mutant is much longer than that of each single mutant. Mean lifespan [days $\pm$ SEM (n)] of worms is as follows: wild type, 22.8 ± 0.38 ($n = 81$); *spr-3(by108)*, 25.5 ± 0.48 ($n = 102$), $P < 0.0001$; *daf-2(e1370)*, 36.1 ± 1.18 ($n = 84$), $P < 0.00001$; and *spr-3(by108);daf-2(e1370)*, 53.8 ± 0.99 ($n = 64$), $P <$ 00.00001, $^{**}P < 0.01$. wt, wild type.

Dauer formation is another classic phenotype of IIS pathway mutant. When monitored at 25°C, both *spr-3(by108);daf-2(e1370)* double mutant and *daf-2(e1370)* mutant formed 100% dauer. When monitored at 21°C, *spr-3(by108);daf-2(e1370)* double mutant formed a little more dauer than mutant *daf-2(e1370)* (**Table 2**). This result also indicated that *spr*-3 truncated mutation enhances the phenotype of *daf-2* deficiency.

Mutant *spr-3(by108)* exhibits increased resistance to environmental stresses

In many cases, *C. elegans* uses common pathways to regulate longevity and stress resistance. Thus, we tested whether the *spr-3(by108)* mutant showed the enhanced resistance to environmental stress stimuli. Adult wild-type or *spr-3(by108)* mutant was treated with 100 mM paraquat for certain time. The survival of each genotype was counted to assay their resistance to acute oxidative stress. Results showed that the *spr-3(by108)* mutant is considerably more resistant to the paraquat treatment compared with wild-type worms. Similarly, the phenotype is also dependent on the *daf-16* function [**Fig. 4(A)**]. The *daf-2(e1370);spr-3(by108)* double mutant showed the enhanced resistance to 50 mM paraquat treatment than each single mutant [**Fig. 4(B)**]. An enhanced resistance to heavy metal exposure was also demonstrated in *spr-3(by108)* mutant [**Fig. 4(C,D)**].

Table 2 Effect of *spr-3* mutation on dauer phenotype

Temperature (°C)	Strain	Total number	Percentage of dauer
21	Wt	132	0
	spr-3(by108)	146	0
	daf-2(e1370)	160	0.23
	daf-2(e1370);spr-3(by108)	109	0.30
25	Wt	215	0
	spr-3(by108)	85	0
	daf-2(e1370)	114	100
	daf-2(e1370);spr-3(by108)	138	100

wt, wild type.

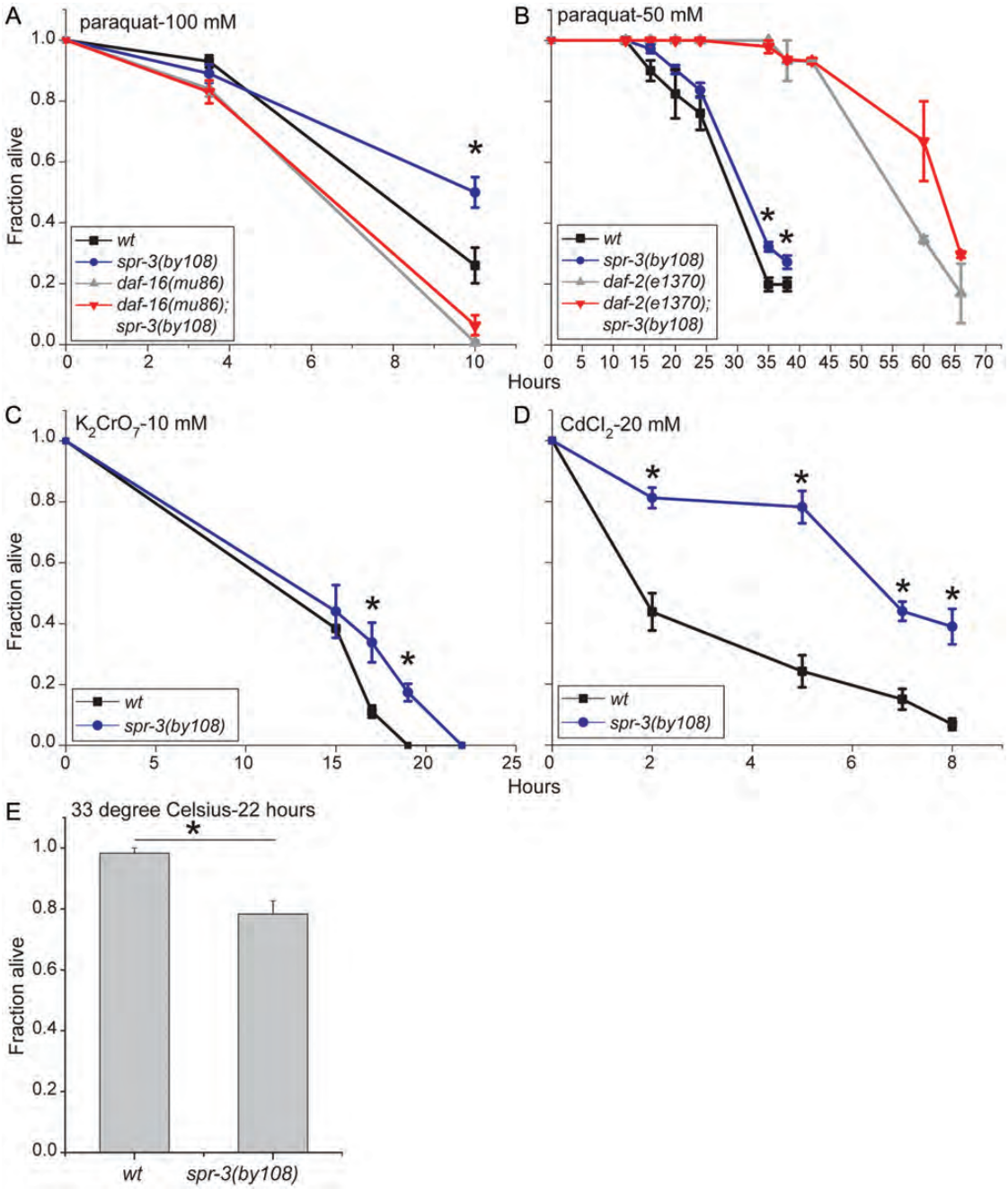

Figure 4 Mutant *spr-3(by108)* showed increased resistance to several environmental stresses (A) The increased resistance to the paraquat was dependent on the *daf-16* function. (B) The increased resistance to the paraquat was enhanced by the IIS pathway mutation. (C,D) Increased resistance to heavy metals (K_2CrO_7 and $CdCl_2$) exposure. (E) Reduced heat tolerance at 33°C in mutant *spr-3(by108)*. *$P < 0.05$. $n > 30$. wt, wild type.

However, *spr-3(by108)* mutant showed less resistance to heat shock at 33°C [**Fig. 4(E)**].

Expression of *daf-16* was up-regulated in *spr-3(by108)* mutant

Since the phenotype of *spr-3(by108)* is abolished by *daf-16* mutation, we then examined the expression of *daf-16* and its target genes *sod-3*, *mtl-1*, and *sip-1* in *spr-3(by108)* mutant. The *sod-3* encodes Fe/Mn superoxide dismutase with 86.3% amino acid sequence identity to its mammalian ortholog. Up-regulation of *sod-3* gene expression was reported to increase the lifespan and stress resistance in *C. elegans* [22–24]. The *mtl-1* encodes metallothionein that responses to cadmium, mercury, and other heavy metal exposure [25,26]. The *sip-1* encodes a stress-induced protein that plays a role in lifespan extension and enhancement of stress resistance. The result of qRT-PCR indicated that the expression of *daf-16* is significantly increased in mutant nematode

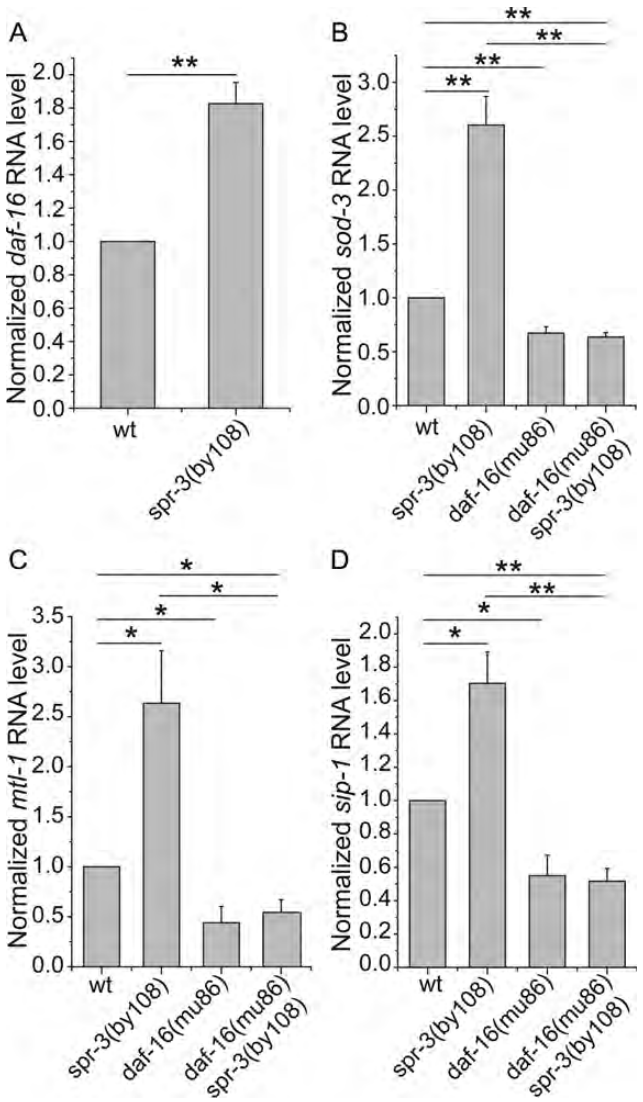

Figure 5 The expression levels of *daf-16* and its target genes were up-regulated in the mutant *spr-3(by108)* (A) Increased expression of *daf-16* in mutant *spr-3(by108)*. Increased expression of *daf-16* target genes *sod-3* (B), *mtl-1* (C), and *sip-1* (D) in mutant *spr-3(by108).* The increased expression of *sod-3*, *mtl-1* and *sip-1* in the *spr-3* mutant was completely dependent on the function of *daf-16*. $*P < 0.05$; $**P < 0.01$. $n > 150$. wt, wild type.

[**Fig. 5(A)**]. Meanwhile, *sod-3*, *mtl-1*, and *sip-1* were up-regulated in *spr-3(by108)* mutant and down-regulated in *daf-16(mu86);spr-3(by108)* double mutant [**Fig. 5(B–D)**].

Discussion

In this study, we showed that *spr-3*, one of the *NRSF/REST* homologue gene in nematode, regulates the lifespan of *C. elegans*. Deletion of *spr-3* gene or RNAi inhibition decreased the lifespan of *C. elegans*. However, *spr-3(by108)* shows long lifespan phenotype and increased resistance to environmental stress. Furthermore, this mutation can dramatically increase the lifespan of *daf-2(e1370)* mutant up to 54 days, ~250% of that in wild-type worms. We demonstrated that the phenotype of mutant *spr-3(by108)* is a gain-of-function mutation. The mutant gene could encode a predicted C-terminal truncated spr-3 protein with a length of 464 amino acids, missing 219 amino acids at C-terminal. This mutant protein contains five N-terminal zinc fingers but missing two zinc fingers at C-terminal [**Fig. 6(A)**], which shares a similar structure of REST4, a neuronal alternative splicing form of NRSF/REST in mammalian [**Fig. 6(B,C)**]. Mammalian REST4 is specifically expressed in adult neurons that can competitively bind to NRSE and hind NRSF/REST transcriptional repression activity [27]. However, the function of REST4 is not clear in mammals. Our data suggested that REST4 plays a potential role in the aging process.

The longevity of mutant *spr-3(by108)* is dependent on the normal function of *daf-16.* The mechanism can be partly explained by up-regulation of *daf-16* and its target genes *sod-3*, *mtl-1*, and *sip-1* in the mutant. The up-regulation of *daf-16* pathway could also be used to explain the increased resistance of *spr-3(by108)* mutant to paraquat treatment and heavy metal exposure. Another interesting finding is that the lifespan extension of *spr-3(by108)* mutant was abolished by *glp-1* mutation. This result indicated that the function of *spr-3(by108)* needs normal germline signaling. In other words, the long lifespan phenotype caused by germline signaling deficiency is also dependent on the normal function of *spr-3*. The expression of *daf-2* in *spr-3(by108)* is significantly down-regulated; however, the double mutant of *spr-3(by108);daf-2(e1370)* showed extremely longer lifespan than each of two single mutants indicating that the longevity function of *spr-3(by108)* is not fully dependent on the inhibition of *daf-2* function, but can synergistically interact with the deficient IIS pathway to up-regulation of *daf-16*.

In summary, our results showed that the *spr-3* plays a role in longevity and stress resistance regulation in *C. elegans*. This result also shed a light on further understanding of the complex function of NRSF/REST in mammalian, especially in aging process and longevity determination. Tissue-specific expression of *spr-3* and the truncated mutant gene, especially in specific neurons in *C. elegans*, and the resulted phenotypes need to be further investigated.

Supplementary Data

Supplementary data are available at *ABBS* online.

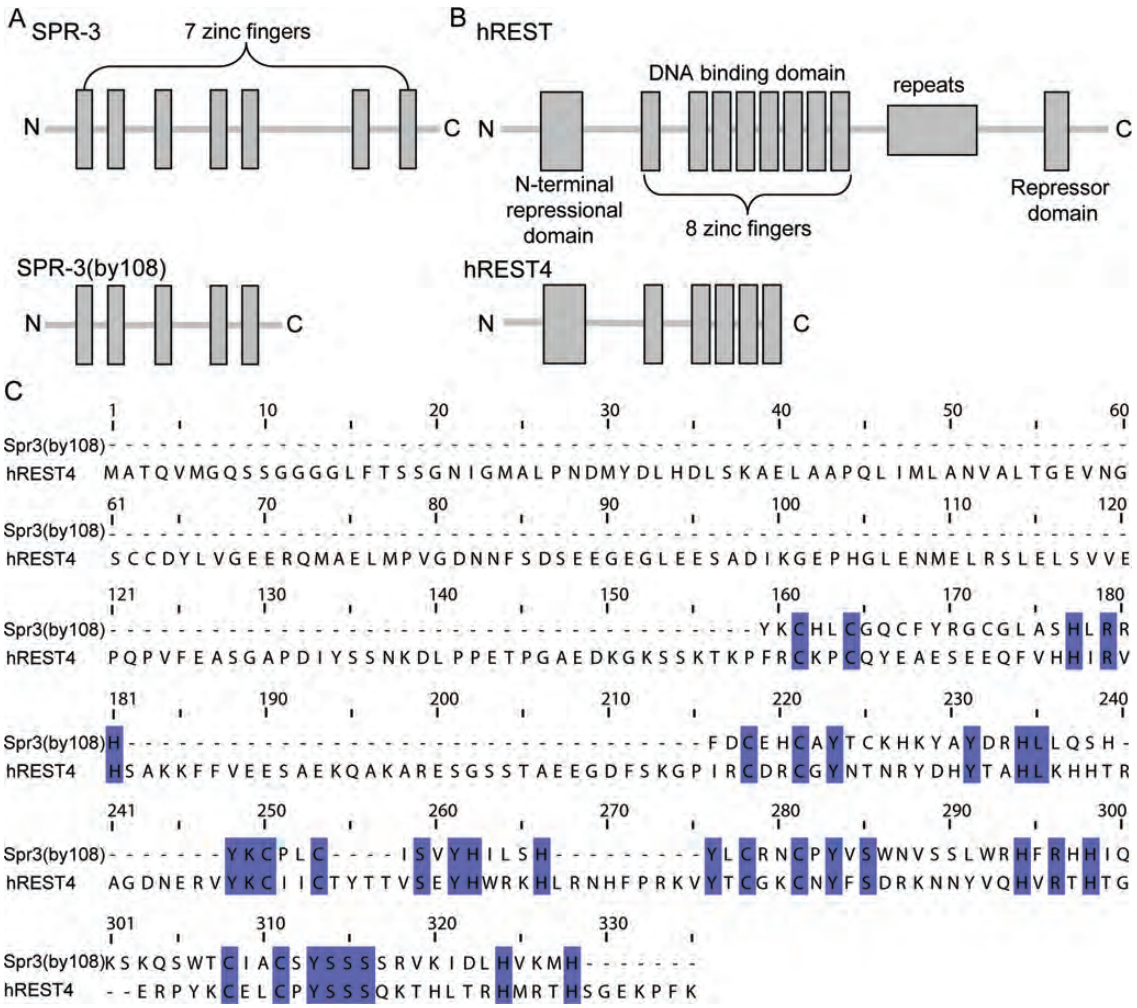

Figure 6 The predicted product of *spr-3(by108)* showed a molecular structure similarity to human REST4 (A) Diagrammatic representation of the modular domain structure of *spr-3* and *spr-3(by108)*. (B) Diagrammatic representation of the modular domain structure of REST and REST4. (C) Protein sequence alignment of the *spr-3(by108)* and hREST4.

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